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10/527,495	05/18/2005	Stephen G Withers	UBC.P-034	4792

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EXAMINER	
RAGHU, GANAPATHIRAM	
ART UNIT	**PAPER NUMBER**
1652	

DATE MAILED: 06/06/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/527,495	WITHERS ET AL.	
	Examiner	Art Unit	
	Ganapathirama Raghu	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *20-41* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *20-23,26,27 and 38-41* is/are rejected.

7)☒ Claim(s) *24,25,28 and 29* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 May 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *03/11/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 20-41 are pending in this application for examination. Claims 20-23, 26-27 and 38-41 are now under consideration. Claims 30-37 are withdrawn as they are drawn to non-elected invention.

Election/Restrictions

Applicants' election of Group I, claims 20-29 and as a species β-glycosidase of *Cellulomonas fimi* Cex E127A with traverse for prosecution in the reply filed on May 15, 2006 is acknowledged. The traversal is on the grounds that the unity of invention exists between the restricted groups and all the claims are closely related and examination of all the claims will not pose a serious search burden.

Applicants arguments of 1). No lack of unity was found by the Examiner, 2). The claims of Groups I-III are related as a product (Group I), a process of specifically making the product (Group II) and a means of carrying out the method (Group III) and 3) Restriction between Groups I and IV should be withdrawn as Group IV is drawn to a fusion protein encompassing the mutant enzyme of Group I are answered as follows.

1.The traversal is on the grounds that the claims meet the unity of invention, applicants' arguments have been fully considered but are not deemed persuasive to withdraw the restriction requirement previously applied for the reasons stated below.

2. Applicant's argument of the claims of Groups I-III are related as a product (Group I), a process of specifically making the product (Group II) and a means of carrying out the method (Group III) and the traversal is on the grounds that the Office has not provided sufficient reasons for restriction of different groups and therefore restriction between groups be withdrawn and

have requested for examination of all the claims. However, MacLeod et al., (Biochemistry, 1994, Vol. 33: 6371-6376), teaches the isolation and characterization of β-glycosidase mutants of Cex from *Cellulomonas fimi* in which Glu 127 is replaced by Asp, Ala and Gly and conclude that the properties of the mutants are consistent with Glu 127 being acid/base catalyst in Cex (Introduction section, last paragraph, column 2, page 6372). Therefore the technical features linking the inventions of Groups I-III does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art. Furthermore searching the polypeptides and the method of use of the polypeptides are not coextensive. Group I polypeptides encompasses molecules which are claimed in terms of mutants and variants of various enzymes with different activities and also from different sources i. e., microorganisms, the search of method of use of all the polypeptides as in group I would involve text search of all the claimed enzymes and would be burdensome and moreover said process or method of use can be carried out by polypeptides that are similar only in activity but from different source and posses different structural features. Therefore, for the above cited reasons searching of all claims is a serious search burden and contrary to applicant's argument, the requirement is still deemed proper and is therefore made FINAL. Further evidence that the claims lack special technical feature is found under U.S.C. 102 (b) and 103 below.

3. Applicants' argument that restriction between Groups I and IV should be withdrawn as Group IV is drawn to a fusion protein encompassing the mutant enzyme of Group I. Examiner considers the argument to be persuasive and therefore restriction of group IV, claims 38-41 is withdrawn and will be considered for examination along with Group I, claims 20-23 and 26-27.

Priority

This application is 371 PCT/CA03/01398 filed on 09/12/2003 which claims the benefit of US Provisional application 60/410502 filed on 09/12/2002. However examiner notes that the elected Group I, claims 20-23, 26-27, and 38-41 and as a species β-glycosidase of *Cellulomonas fimi* Cex E127A were not disclosed either in the Provisional application 60/410502 filed o 09/12/2002. Therefore the priority date for said elected claims is effectively the filing date 371 PCT/CA03/01398 filed on 09/12/2003.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 11 March 2005 is in compliance with the provisions of 37 CFR 1.97. Accordingly, examiner is considering the information disclosure statement.

Drawings

Drawings are accepted for examination purposes only.

Claim Objections

Claims 23 and 40-41 are objected to, as they are drawn to non-elected species of inventions. Appropriate correction is required.

Claim Rejections 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 21 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 26, 39 and 40 recites the phrase "...smaller than the smaller chain...". This sentence is confusing as an amino acid has only a single side chain. The examiner suggests amending the claim to recite "...smaller than that of..." Clarification is required.

Claim 22 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 22 is confusing as many of the amino acids side chains listed are not smaller or equal in size to Asp or Glu as recited in claim 21 from which claim 22 depends. Correction is required.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 20 and claims 21-23 depending therefrom and claim 38-39 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 20-23 are directed to a mutant glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid

side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline, phenylalanine and tyrosine. Claims 38 and 39 are directed to a mutant glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain and a binding element for immobilization of the fusion protein. Claims 20-23 and 38-39 are rejected under this section 35 U.S.C. 112, because the claims are directed to a mutant glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline,

phenyalanine and tyrosine and said mutant enzyme in addition has a binding element for immobilization of the fusion protein, because the mutant enzyme involves a genus of polypeptides from any or all sources with no support in the specification for the structural details associated with the function i.e., β-glycosidase activity and in addition said mutant enzyme is a fusion protein with a genus of binding elements from any or all sources serving as the binding element in the fusion proteins. No description of identifying characteristics of all of the sequences of an isolated mutated polypeptide from any or all sources having β-glycosidase activity having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline, phenyalanine and tyrosine, wherein said mutant enzyme in addition has a binding element as a fusion protein form any or all sources of enzymes has been provided by the applicants in the specification. No information, beyond the characterization of an endo-acting retaining β-glycosidase of *Cellulomonas fimi* and said mutant enzyme is Cex E127 or an *Agrobacterium* β-glucosidase said mutant enzyme is Abg E171 or an endo-mannanase Man26A of *Cellvibrio japonicus* said mutant is Man26A E212A, and said mutants are fusion proteins consisting the cellulose-binding domain of *Cellulomonas fimi* exoglucanase, has been provided by the applicants, which would indicate that they had possession of the claimed genus of the

polypeptides, isolated mutated polypeptide from any or all sources having glycosidase activity, said enzyme having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline, phenyalanine and tyrosine, wherein said mutant enzyme in addition has a binding element as a fusion protein form any or all sources of enzymes has been provided by the applicants in the specification. Therefore, one skilled in the art cannot reasonably conclude that applicant had possession of the claimed invention at the time the instant application was filed. Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claims 20-23 and 38-39 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a mutant enzyme of an endo-acting retaining β-glycosidase of *Cellulomonas fimi*, does not reasonably provide enablement for any mutant β-glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base

catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline, phenyalanine and tyrosine and said mutant enzyme in addition has a binding element for immobilization of the fusion protein. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention commensurate in scope with the claim.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 20-23 and 38-39 are so broad as to encompass a mutant β-glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different

amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline, phenyalanine and tyrosine and said mutant enzyme in addition has a binding element for immobilization of the fusion protein, because the mutant enzyme involves a genus of polypeptides from any or all sources with no support in the specification for the structural details associated with the function i.e., β-glycosidase activity and in addition said mutant enzyme is a fusion protein with a genus of binding elements from any or all sources serving as the binding element in the fusion proteins. The scope of the claims are not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polypeptides broadly encompassed by the claims. Since the amino acid sequence of a protein encoded by a polynucleotide determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires knowledge and guidance with regard to which amino acids in the protein's sequence and the respective codons in its polynucleotide, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the encoded proteins' structure relates to its function. However, in this case the disclosure is limited to a mutant endo-acting retaining β-glycosidase of *Cellulomonas fimi* and said mutant enzyme is Cex E127 or an *Agrobacterium* β-glucosidase said mutant enzyme is Abg E171 or an endo-mannanase Man26A of *Cellvibrio japonicus* said mutant is Man26A E212A, and said mutants are fusion proteins consisting the cellulose-binding domain of *Cellulomonas fimi* exoglucanase, but provides no guidance with regard to the making of other mutants and other binding element domains in the fusion protein from any or all sources. In view of the great breadth of the claims, amount of

experimentation required to make the claimed polypeptides the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (e.g., see Ngo et al. in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495), the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by this claim.

While enzyme isolation techniques, recombinant and mutagenesis techniques are known, and it is not routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions or deletions.

The specification does not support the broad scope of the claims for a mutant enzyme β-glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine,

isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline, phenyalanine and tyrosine and said mutant enzyme in addition has a binding element for immobilization of the fusion protein, because the mutant enzyme involves a genus of polypeptides from any or all sources with no support in the specification for the structural details associated with the function i.e., glycosidase activity and in addition said mutant enzyme is a fusion protein with a genus of binding elements from any or all sources serving as the binding element in the fusion proteins, because the specification does not establish: (A) catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme from any or all sources, including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid without affecting the activity of encoded β-glycosidase activity, wherein said different amino acid for modification is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asapragine, glutamine, histidine, proline, phenyalanine and tyrosine; (B) the general tolerance of the polypeptide and the polynucleotide encoding glycosidase activity to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue or the respective codon in the polynucleotide with an expectation of obtaining the desired biological function; (D) said mutant enzyme is a fusion protein which has a binding element for immobilization of the fusion protein, said binding element is from any or all sources; and (E) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claim broadly including methods of using polypeptides with an enormous number of modifications. The scope of the claim must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of polypeptides i.e., a mutant glycosidase, said enzyme being selected from among glycosidase enzymes from any or all sources having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asparagine, glutamine, histidine, proline, phenyalanine and tyrosine and said mutant enzyme in addition has a binding element for immobilization of the fusion protein, because the mutant enzyme involves a genus of polypeptides from any or all sources with no support in the specification for the structural details associated with the function i.e., β-glycosidase activity and in addition said mutant enzyme is a fusion protein with a genus of binding elements from any or all sources serving as the binding element in the fusion protein, is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 20-23 and 26-27 are rejected under 35 U.S.C. 102(b) as being anticipated by Claims 20-23 and 26-27 are directed MacLeod et al., (Biochemistry, 1994, Vol. 33: 6371-6376). a mutant enzyme β-glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asparagine, glutamine, histidine, proline, phenylalanine and tyrosine, said mutant enzyme is a mutant of an endo-acting retaining β-glycosidase of *Cellulomonas fimi* and is Cex E127A. MacLeod et al., teach the wild-type exoglycanase/xylanase Cex from *Cellulomonas fimi* a retaining β-glycosidase, hydrolyzes β-1,4 glycosidic bonds with net retention of anomeric configuration, releasing the disaccharides β-cellobiose and β-xylobiose. The enzyme uses a double displacement mechanism involving a glycosyl-enzyme intermediate which is formed and hydrolyzed with general acid/base catalytic

assistance and Glu127 was proposed as the acid/base catalyst on the basis of sequence alignments and mutants in this position were constructed in which the glutamic acid was replaced by alanine or glycine (Abstract section, page 6371). Said reference also teaches the isolation and characterization of mutants of Cex in which Glu 127 is replaced by Asp, Ala and Gly and conclude that the properties of the mutants are consistent with Glu 127 being acid/base catalyst in Cex (Introduction section, last paragraph, column 2, page 6372). Therefore, MacLeod et al., anticipate claims 20-23 and 26-27 as written.

Claims 20-23 and 26-27 are rejected under 35 U.S.C. 102(b) as being anticipated by MacLeod et al., (Biochemistry, 1996, Vol. 35: 13165-13172). Claims 20-23 and 26-27 are directed a mutant enzyme β-glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asparagine, glutamine, histidine, proline, phenylalanine and tyrosine, said mutant enzyme is a mutant of an endo-acting retaining β-glycosidase of *Cellulomonas fimi* and is Cex E127A. MacLeod et al., teach the isolation of a mutant form Cex E127A of exoglycanase/xylanase Cex from *Cellulomonas fimi* a retaining β-glycosidase (Abstract section, page 13165). Said reference also teaches the isolation and

characterization of mutants of Cex in which Glu 127 is replaced by Ala (Materials and Methods section, first paragraph, column 1, page 13166) and the kinetic properties of the mutant enzyme Cex E127A (Results section, second paragraph, column 2, page 13169) and conclude that the properties of the mutants are consistent with Glu 127 being acid/base catalyst in Cex. Therefore, MacLeod et al., anticipate claims 20-23 and 26-27 as written.

Claim Rejections: 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 20-23, 26-27 and 38-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over MacLeod et al., (Biochemistry, 1994, Vol. 33: 6371-6376), MacLeod et al., (Biochemistry, 1996, Vol. 35: 13165-13172) and further in view Kilburn et al., (US patent 5,137,819, date of

patent 08/11/1992) and Park et al., (Methods in Enzymology, 2000, Vol. 326: 418-429). Claims 20-23, 26-27 and 38-41 are directed to a mutant enzyme β-glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, glycine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, asparagine, glutamine, histidine, proline, phenylalanine and tyrosine, said mutant enzyme is a mutant of an endo-acting retaining β-glycosidase of *Cellulomonas fimi* and is Cex E127A, wherein said mutant enzyme in addition has a binding element as a fusion protein, said binding element is cellulose-binding domain of *Cellulomonas fimi* exoglucanase.

The references of MacLeod et al., (Biochemistry, 1994, Vol. 33: 6371-6376), MacLeod et al., (Biochemistry, 1996, Vol. 35: 13165-13172) as they apply to claims 20-23 and 26-27 has already been discussed above (see rejection under 35 U.S.C. 102 (b)). While the references of MacLeod et al., (1994) and MacLeod et al., (1996) (*supra*) teach the wild-type exoglycanase/xylanase Cex from *Cellulomonas fimi* a retaining β-glycosidase, hydrolyzes β-1,4 glycosidic bonds with net retention of anomeric configuration, releasing the disaccharides β-cellobiose and β-xylobiose and a mutant enzyme β-glycosidase, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of wild-type enzyme including a catalytically active amino acid

acting as an acid, base, or acid/base catalyst, said mutant enzyme being mutated to replace the catalytically active amino acid acting as an acid, base or acid/base catalyst with a different amino acid having a non-carboxylic acid side chain that is approximately equal in size to or smaller than the chain of the replaced amino acid, wherein said different amino acid is selected from alanine, said mutant enzyme is a mutant of an endo-acting retaining β-glycosidase of *Cellulomonas fimi* and is Cex E127A. Said references do not explicitly teach said mutant enzyme as a fusion protein having a binding element, said binding element is cellulose-binding domain of *Cellulomonas fimi* exoglucanase.

Kilburn et al., and Park et al., (*supra*), teach the fusion proteins containing a polypeptide such as an enzyme and a substrate binding element such as cellulose binding domain and the use of the fusion protein for isolation and purification on affinity matrix columns. Said two references teach both amino and carboxy terminus fusion proteins consisting the cellulose binding domains and specifically Kilburn et al., teach the cellulose-binding domain of *Cellulomonas fimi* exoglucanase (entire document).

The instant application relates to engineered β-glycosidase enzymes as fusion proteins having cellulose binding domain as the affinity tag to purify the proteins of interest, and their use for the synthesis of thioglycosides and applications thereof.

Combining the teachings of the above references, it would have been obvious to one of ordinary skill in the art at the time of the instant invention to develop a mutant enzyme β-glycosidase, said mutant enzyme is a mutant of an endo-acting retaining β-glycosidase of *Cellulomonas fimi* and is Cex E127A as taught by MacLeod et al., (Biochemistry, 1994, Vol. 33: 6371-6376), MacLeod et al., (Biochemistry, 1996, Vol. 35: 13165-13172).

One of ordinary skill in the art would have been motivated to make or use such a mutant enzyme β-glycosidase with cellulose binding domains as fusion protein, as the cellulose binding domain can be used as affinity tags for efficient purification and enrichment of fusion proteins. One of ordinary skill in the art would have had a reasonable expectation of success, since the references of MacLeod et al., (Biochemistry, 1994, Vol. 33: 6371-6376), MacLeod et al., (Biochemistry, 1996, Vol. 35: 13165-13172) specifically teach the endo-acting retaining β-glycosidase of *Cellulomonas fimi* and is Cex E127A and the references of Kilburn et al., and Park et al., (*supra*) teach the cellulose binding domain of *Cellulomonas fimi* exoglucanase and the use of the fusion protein for isolation and purification on affinity matrix columns.

Therefore, the above references render claims 20-23, 26-27 and 38-41 *prima facie* obvious to one of ordinary skill in the art.

Conclusion

None of the claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ganapathirama Raghu whose telephone number is 571-272-4533. The examiner can normally be reached on 8 am - 4.30 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300 for regular communications and for After Final communications.

Any inquiry of a general nature or relating to the status of the application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-

direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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May 25, 2006.

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